PROTHORACIC GLANDS OF ADULT LEPIDOPTERA

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INTRODUCTION

The presence of gland-like pouches on the prothorax of certain Lepidoptera has long been known, but the general nature of these structures has never been clearly determined. It is the purpose of this paper to inquire into the nature of the glands and their secretion, and to ascertain some facts concerning their function. The writer wishes to express appreciation to Prof. A. B. Dawson for his help and suggestions concerning the histology.

EARLIER WORK

Degeer (1752, 1778) was the first to record the presence of prothoracic glands in adult Lepidoptera. He observed the secretion of two drops of clear transparent liquid from the dorsal anterior region of the thorax (*Arctia caia* L.) and figured the approximate location of these drops. These were secreted when the moth was disturbed. Degeer believed this to be an original observation. Zeller (1840) stated that beneath the neck collar (*Arctia caia*) were two red tufts of hair which upon irritation of the moth became visible. These hairs clothed the glands which opened sideways. A drop of yellow liquid could be squirted out to a distance or could be reabsorbed. Squirting occurred not more than twice. Zeller thought the supply was then exhausted. The liquid possessed an odor similar to that of coccinellids or fresh fluid from poppy stalks and tasted as sharp as the former. Fenn (1890) reported the secretion of pale greenish or yellowish fluid from the dorsal anterior portion of the thorax of *Liparis salicis* L. (*Stilpnotia salicis* L.) when the animal was annoyed. Griffiths (1890) in reporting the same for *Arctia caia* added that the glands were whitish processes larger at the orifice than below, and the fluid gave an acid reaction with litmus. He postulated that the fluid was for defense since the moths were distasteful to birds. Webb (1890) reported that the glands occurred in many Cuspi-

dates (this probably refers to *Apatela*) and probably secreted a form of formic acid. Reid (1891) reported their presence in cocoon forming Bombyces, *Dicranura,* and also *Viminia myricæ* Gúen. (*Apatela myricæ* Gúen.). He postulated that the secretion aided in emergence from the cocoon (*B. quercus* L. (*Lasiocampa quercus* L.) or *D. vinula* L.) since the cocoon just before emergence was moist in this area, and the glands were flowing freely. The secretion was most plentiful at emergence and diminished with age. Portschinsky (1892) reported the odor (*Arctia caia*) as resembling that of coccinellids. He figured the attitude of the moth in the act of secreting and inferred that this was a protective device. He is quoted by Schulze (1912) as saying that no secretion occurred in *Arctia villica* L., since the white thoracic spots imitated the protective secretion. Uffeln (1909) described the secretion from the prothorax of a female (*Arctia caia*) as being an oily yellow, watery clear fluid smelling like nettle. He postulated that it was either repellent or attractant in nature. Dampf (1909) figured the location of the glands with reference to the patagia. The patagia were elevated by the entrance of blood into the membranous parts of the thorax. He reported the glands in Zygænids and *Callimorpha dominula* L. Hollande (1911) defined the location of vesicles with reference to the patagia (*Stilpnotia salicis* L.). These vesicles already filled with blood were filled with more. With sufficient pressure there arose on the internal angle of each a drop of liquid. *Hyponomeuta* (syn. *evonymellus* Scop.) *cognatellus* Hubner emitted blood from the side edges of the neck due to the rupture of a small vesicle. *Argyresthia nitdella* Fab. secreted under the patagia also by means of a small rupture. In *Spilosoma menthastri* Esp., *Lithosia griseola* Hubner, *Hypocrita jacobeæ* L., and *Arctia flavia* Fuessl., secretion occurred as in *S. salicis* L. *A. flavia* Fuessl., threw two orange jets more than twenty centimeters. *Zygæna trifolii* Esp., gave forth blood at the articulations of the coxæ with the thorax due to ruptures. Hollande stated that in the above named species the fluid emitted was definitely blood in contrast to the case of *Spilosoma fuliginosa* L. (*Phragmatobia fuliginosa* L.) where there were true glands under the patagia which took the place of the blood vesicles. These glands were bifurcate and pyriform. Hollande pricked a male

moth and found that the glands could no longer be elevated. When the blood coagulated in the wound or the wound was closed with celloidin, the glands elevated normally. In this way he proved that the pressure of the blood in all cases which he examined was responsible for the elevation of the glands and the blood vesicles. He further proved by decapitating *Stilpnotia salicis* L., with a ligature to prevent the loss of blood that the action of the glands and vesicles was reflex. Schulze (1912) figured the glands of *Spilosoma luteum* Hufn., showing their relation to the patagia. He also showed a photomicrograph upon which the aperture of the gland is indicated as being at the inner edge of the patagium. After reviewing the literature (1913) he said that the fluid in a freshly emerged female (*Arctia caia*) was clear as glass on the right side and turbid yellow on the left. After the glands were wiped and squeezed, both secreted yellow. Ten different people were not able to perceive the odor. He thought possibly that the secretion was thrust out first, followed by clear blood; but he did not believe that the blood of *Arctia caia* smelled like coccinellid blood. Schultz (1914) found no glands in the patagia or on the prothorax of *Stilpnotia salicis, Arctia caia,* and *Hyponomeuta evonymella.* Isaak (1916) stated that the glands (*Arctia caia*) were located on the mesothorax and were either light producing or glowing organs since the secretion shone green in the dark. The light beamed for ten seconds and was then withdrawn. It occurred in both males and females. Soldanski (1916) summarized Isaak's and Schulze's papers and said that he was unable to see shining. He reported that many other observers had met with the same failure. Aue (1916) summarized Isaak's paper. Hykes (1917) listed and reviewed a portion of the literature on these glands. Aue (1918, 1918a) described the fluid as being crystal clear and lasting for five seconds before being reabsorbed. He gave the location as on the thorax behind the head. He was unable to observe any shining in the dark. Just (1918) listed the literature on the subject up to 1918. Aue (1922) listed a portion of the literature. He described the liquid as being clear and colorless. It had a very penetrating odor, burned the tongue, yet was not tasty, and was not luminous. He found the glands in males.

ANATOMY

The two glands in *Apantesis arge* Drury lie on the dorsal side of the prothorax and not on the mesothorax as Isaak (1916) stated. Each is essentially a soft membranous pouch lying under and hidden by the patagium. Viewed from the side (Fig. 3A) the gland is seen as a lobe which is continuous with the intersegmental membrane and is bounded anteriorly by the "Spange"

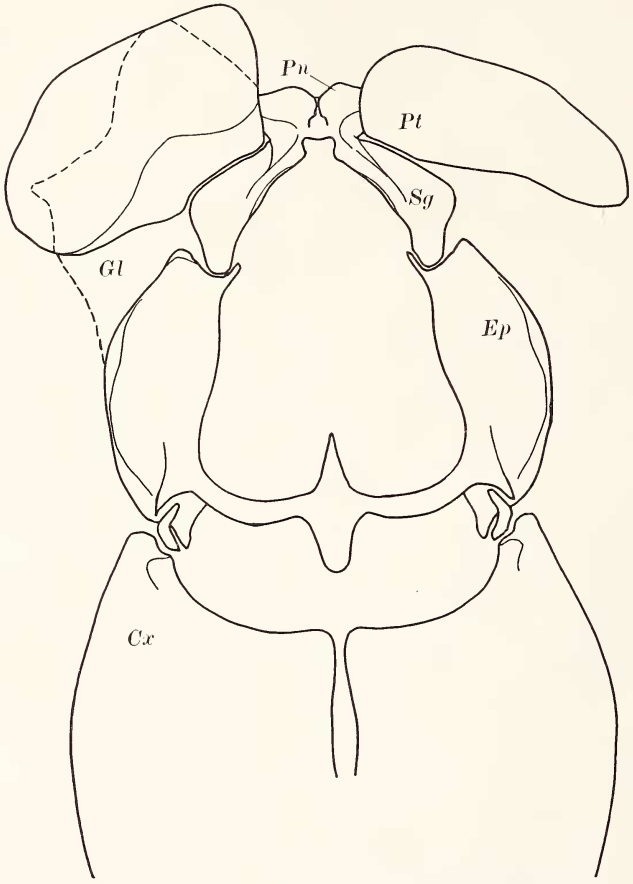

FIG. 1. Front view of the prothorax of *Apantesis arge* showing the relation of the gland to the skeletal parts. The right patagium is raised as in the act of secretion. Pn, pronotum; Pt, patagium; Sg, "Spange" of the episternum; Gl, gland; Ep, episternum; Cx, coxa.

of the episternum and by the patagium. It is bounded laterally by the episternum and on the median side by the pronotum (Fig. 3B). Posteriorly it is limited by the scutum of the mesothorax (Fig. 3A). The mesothoracic spiracle lies ventral and slightly posterior to the gland. When the moth is viewed from the front, the gland is completely hidden by the patagium (Fig. 1). The gland itself is but slightly smaller in area than the patagium (Fig. 3B) and is densely clothed with hairs and scales. Its dorsal surface is directly continuous with the ventral surface of the patagium. On the dorso-lateral surface of each gland is a prominent tit which does not, in this species, lie at the inner edge of the patagium as Schulze (1912) figured for *Arctia caia*. This tit, which marks the aperture, is conspicuous because it is usually heavily pigmented, heavily chitinized, and more thickly clothed with scales than the rest of the gland. Oftentimes a bit of secretion will dry on these very dense hairs and completely block the aperture.

HISTOLOGY

Live males and females of *Apantesis arge* were killed and fixed in ninety-five per cent alcohol. Other fixatives were not available in the field, and this was deemed sufficient for determining general histological details and topographical relationships. The insects were infiltrated with two, four, six, eight, ten, twelve and fourteen per cent celloidin for a period of twenty-four hours each at a temperature of forty-five degrees centigrade. They remained an additional week in fourteen per cent while it thickened. Sections were cut at fourteen and twenty microns. Some were stained with Harris' hematoxylin and eosin; others, with Heidenhain's iron hematoxylin and alum.

The gland is a large pouch formed by the invagination of the hypodermis (Fig. 2). It is not connected directly with the body cavity as Hollande (1911) figured for *Stilpnotia salicis*. Its top and sides are composed of two closely applied layers of cells, the outer being the regular hypodermal cells which secrete the cuticle and among which are the trichogen cells of the scales, the inner being the invaginated portion and forming the internal roof, walls, and bottom of the gland (Fig. 2). Often there is a great deal of blood between these layers. There is also an exceedingly large

quantity of blood beneath the floor of the gland. In no instance were blood cells found within the gland itself, nor was any direct connection with the body cavity observed. The gland epithelium was composed of a single layer of surprisingly thin cells (Fig. 3C). They were so thin, in fact, that the nuclei bulged out, giving the string of cells a bead-like appearance. The nuclei were relatively

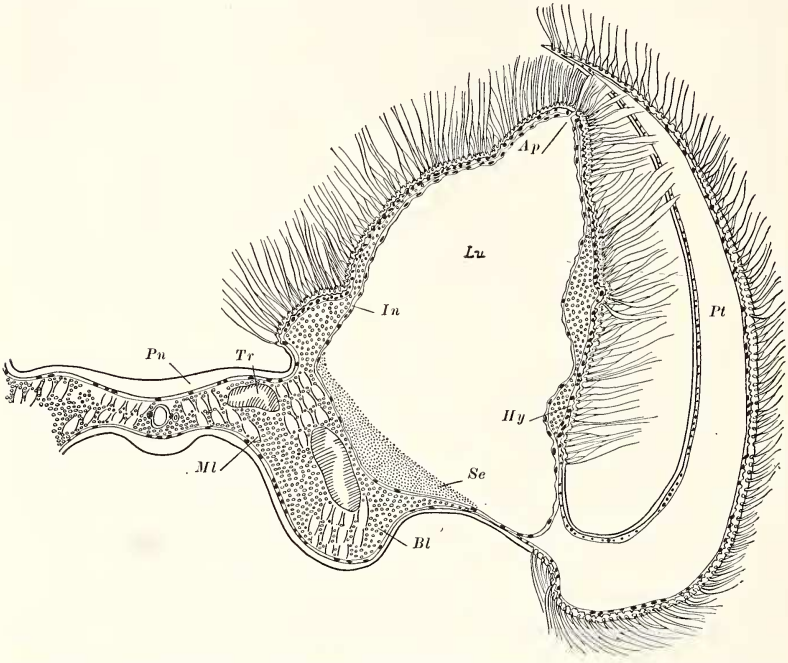

FIG. 2. Transverse section through the prothorax of *A. argе* showing the left gland only. The presence of the patagium in this section is due to its slight lateral overhang. Ap, aperture; Lu, lumen of the gland; In, invaginated cell layer; Hy, hypodermis; Se, secretion; Pt, patagium; Pn, pronotum; Tr, trachea; Ml, muscle; Bl, blood.

large but elongated. They were either solidly filled with chromatin or filled with large coarse granules. The small amount of cytoplasm present appeared to be relatively clear. Lying on top of these cells was an exceedingly thin clear cuticle. This seemed to be greatly folded so that it presented the appearance of many closely lying parallel ridges which were dentate when viewed in profile. In sections cut at right angles to the direction in which

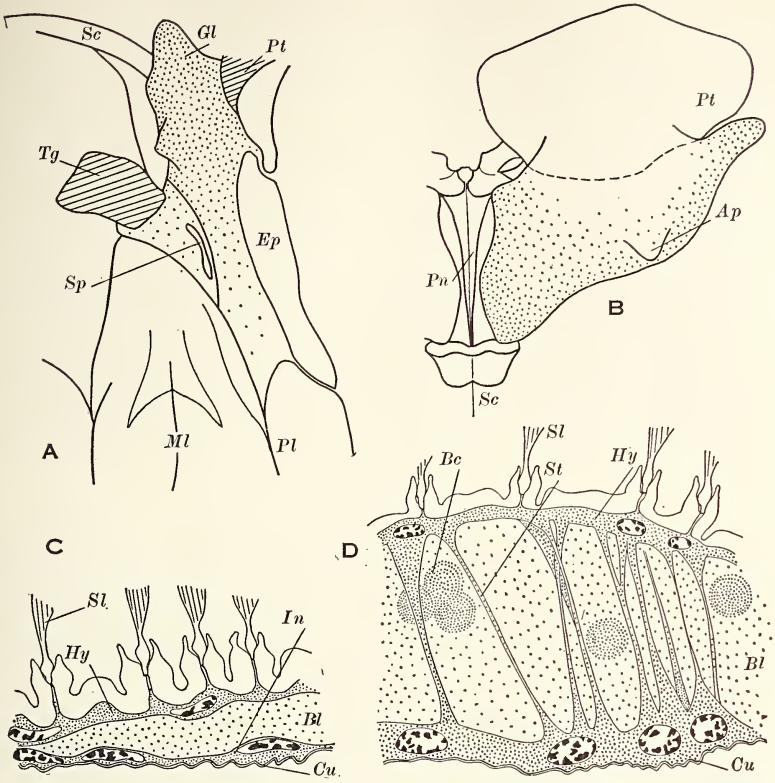

FIG. 3. A. The left side of the pro- and mesothorax of *A. argc* with the patagium and tegula removed. Sc, scutum of the mesothorax; Gl, gland; Pt, location of the patagium; Tg, location of the tegula; Sp, mesothoracic spiracle; Ep, episternum; Pl, prothoracic leg; Ml, mesothoracic leg. B. Dorsal view of the right side of the prothorax showing the patagium raised. Pt, patagium; Pn, pronotum; Ap, gland aperture; Sc, scutum of the mesothorax. C. Cross section of the gland wall of a moth several days old. Sl, scale; Hy, hypodermis; In, invaginated cell layer; Bl, blood; Cu, cuticle. D. Cross section of the gland wall of a freshly emerged moth. St, protoplasmic strand; Bc, blood cell.

the ridges ran, the ridges appeared as many fine short papillæ projecting into the lumen of the gland. Small masses of secreted material were seen close to the gland wall. These consisted of a homogeneous material ranging from finely granular to clear. Except for these the lumen was empty. The moths had been killed after they had secreted several drops.

Upon examination of a freshly emerged specimen which had not so far as was known secreted any liquid, it was found that the gland presented quite a different appearance (Fig. 3D). The distance between the two cell layers of the gland walls was very great. At no point did the two layers touch. The cells of the invaginated layer were slightly larger and more rounded than in the previous case. Their nuclei were also slightly larger and less flattened. Most remarkable, however, was the presence of numerous strands extending from the inner layer to the outer. These strands semed to be cytoplasmic extensions of the inner cells but were morphologically continuous with the cells of the hypodermis. Blood filled the entire area. The patagia, which in other cases were empty, were here divided into sinuses by similar thin strands and membranes which stretched forth from the hypodermis on all sides.

FUNCTION

I first observed these glands while examining a female *Isia isabella* Abbot and Smith which had recently emerged from the cocoon. This specimen secreted four large drops of yellowish fluid from each gland. The last drops secreted were largely air bubbles which were reabsorbed before breaking.

Further detailed study was made upon *Apantesis arge* Drury. The secretion was a clear honey colored liquid which precipitated in ninety-five per cent alcohol as a cloudy white precipitate. It gave no test with litmus or phenolphthalein. It sank in water and was as miscible with water as is ninety-five per cent alcohol, leaving an oily spot on the surface. After a fifteen-minute exposure to air the fluid turned black. Under a high power microscope it was seen to contain many clear irregular particles of very small dimensions. In some instances the secretion possessed a slight musty odor; at other times it was odorless.

In order to determine the amount of liquid a moth was able to secrete, I caused three adults to secrete at definite intervals. The secretion was removed each time. All the data are summarized in Table 1.

Since no further secretion occurred after July 22, all three adults were killed on July 25. During this period of observation the moths were not fed. It was thus possible to compute the total

TABLE 1

Amount of Liquid Secreted at Certain Time Intervals

Time	Moth A	Moth B	Moth C
July 20	Three drops from each gland		Two drops from each gland
July 21			
6: 00 P.M.	No secretion (33 hrs. after emergence)	Two drops from each gland	Two drops
9: 00 P.M.	Four drops	Two drops	One drop
9: 20 P.M.	One drop (36 hrs. after emergence)	Two drops	Two drops
9: 30 P.M.	Two drops	No secretion	No secretion
9: 45 P.M.	One very small drop	No secretion	No secretion
July 22			
9: 45 A.M.	One very small drop (mostly air)	No secretion	No secretion
6: 45 P.M.	One drop	One drop	One drop
July 23			
Noon	No secretion	No secretion	No secretion
July 24			
1: 30 P.M.	No secretion	No secretion	No secretion
Total from one gland	Twelve full sized drops	Seven drops	Eight drops

secretion of an animal which had taken in no liquid. Two of the drops that Moth A secreted were very minute and are counted as one thus giving a total of twelve drops from one gland or twenty-four from both. The minimum diameter of the drops was 1 mm. The volume of one drop was thus .00052 cc. Since there were twenty-four drops, the total secretion of the animal was .012 cc. Whether this figure would differ greatly from that of an animal which was allowed to feed is not known.

The maximum number of drops secreted at any one time from one gland was four; these were equal to a volume of .002 cc. The approximate volume of a gland as computed from a whole mount and sections was .002 cc. Thus it may be concluded that the capacity of the gland is very roughly equal to .002 cc. From Table 1 it may be seen that from three to twelve hours time is required to just partially replenish the supply.

No secretion could be obtained from a moth in the act of emerg-

ing; nor did it secrete till two hours afterwards. Other specimens which were tested a few hours after emergence secreted abundantly. One moth lived for eight days and was capable of secreting up to the time of death.

Secretion occurred only when the animal was disturbed by being touched at some point on the body. In one case, however, an individual secreted when the cover of the breeding cage was roughly lifted. Hollande (1911) observed that *Arctia flavia* threw the liquid a distance of twenty centimeters. This was also observed in *Isia isabella* but not in *Apantesis arge*. When the moth was left to itself, the patagia returned passively to their normal position (they were raised to allow secretion) (Figs. 1, 3B); and after ten minutes the secretion was reabsorbed by the glands. Even in one instance when the fluid had already turned black, it was drawn back.

Reid (1891) suggested that the secretion aided in emergence from the cocoon since just before emergence the cocoon was moist and the glands flowing freely. In *Apantesis arge* there is no cocoon to speak of, and the glands were not secreting at the time of emergence. Griffiths (1890) believed that the glands were protective devices. Uffeln (1909) said they were either repellent or attractant. I am inclined to favor Griffiths' interpretation especially in view of the fact that the glands may remain active during the whole lifetime of the individual and never function unless the moth is disturbed. Since both sexes possess these structures, I do not believe them to be attractant in function. Moreover, the secretion from *Isia isabella* and *Apantesis arge* is not luminous.

Up to the time of writing it has been definitely determined that these glands and vesicles occur in the following Lepidoptera: *Arctia caia* L. (Degeer, 1752, 1778), *Stilpnotia salicis* L. (Fenn, 1890), many cuspidates (Webb, 1890), cocoon forming Bombyces, *Dicranura vinula* L., *Viminia myricæ* Gúen., and *Lasiocampa quercus* L. (Reid, 1891); zygænids and *Callimorpha dominula* L. (Dampf, 1909); *Hyponomeuta cognatellus* Hubn., *Argyresthia nitidella* Fab., *Spilosoma menthastri* Esp., *Spilosoma fuliginosa* L., *Lithosia griseola* Hubn., *Hypocrita jacobeæ* L., and *Arctia flavia* Fuessl. (Hollande, 1911); and *Spilosoma luteum* Hufn. (Schulze,

1912). I have observed the glands in the following species: *Apantesis arge* Drury, *Apantesis virguncula* Kirby, *Isia isabella* Abbot and Smith, *Estigmene acræa* Drury, *Diacrisia virginica* Fab., and *Ecpantheria deflorata* Fab. The following dried specimens were heated in ten per cent potassium hydroxide and examined for the presence of glands. The type of structure found, if any, is described; but it is not definitely maintained that these are glands. The living animals or sections of preserved material must be examined for the final decision. The following list is arranged according to the Barnes and McDunnough Check List (1917).

Syntomidæ	
Ctenucha virginica Charp.	Pouches poorly developed.
Arctiidæ	
Hypoprepia miniata Kirby	Moderately developed pouches.
Hemilhaylæ edwardsi Packard	Well developed pouches.
Halisidota maculata Harris	Very poorly developed pouches.
Halisidota tessellaris A. & S.	No well developed pouches.
Holomelina opella Grote	Well developed pouches.
Holomelina diminutiva Graef.	Well developed pouches.
Leptarctia dimidiata Streck.	Rather well developed pouches.
Hyphoraia parthenas Harris	Well developed pouches with a small chitinized tit on the inner margin of one.
Phragmatobia fuliginosa L.	Well developed slightly bifurcate pouches.
Mænas vestalis Packard	Small pouches.
Euerythra trimaculata Smith	Very poorly developed pouches.
Hyphantria textor Harris	Very small poorly developed pouches.
Arachnis picta Packard	Well developed pouches each with a small chitinized tit.
Apantesis persephone Grote	Well developed pouches.
Apantesis radians Walker	Well developed pouches.
Apantesis phalerata Harris	Well developed pouches.
Pygarctia abdominalis Grote	Well developed pouches.
Euchætias egle Drury	No well developed pouches.
Seirarctia echo A. & S.	Well developed pouches.
Turuptiana permaculata Packard	Very small poorly developed pouches.
Parasemia plantaginis L.	Well developed with a chitinized tit on each.

Platyprepia virginalis Bdv.	Well developed pouches with chitinized ridges and no aperture evident.
Utetheisa bella L.	Well developed pouches.
Haploa colona Hubner	Well developed pouches.
Haploa lecontei Bdv.	Well developed pouches.
Haploa milataris Harris	Well developed pouches.
Agaristidæ	
Alypia octomaculata Fab.	No pouches.
Noctuidæ	
Autographa brassicæ Riley	No pouches present.
Notodontidæ	
Datana ministra Drury	Pouches poorly developed.
Lymantriidæ	
Porthetria dispar L.	Well developed pouches.
Lasiocampidæ	
Malacosoma americanum Fab.	No pouches present.

Without examining living material and fresh secretions one can not say definitely in how many forms prothoracic glands occur. Whether Hollande's blood vesicles are distinct from glands remains to be seen. As far as can be seen from Figure 3A the true gland extends as far down as the coxa. Whether the emission of blood from *Zygæna trifolii* Esp. at the articulation of the coxæ with the thorax is from a vesicle comparable to that in other forms also awaits determination. The occurrence of prothoracic pouches is very widespread in the Arctiidæ. Secretion of fluid from the region of the patagia has been reported from Lasiocampidæ, Lymantriidæ, Zygænidæ, Noctuidæ, Notodontidæ, and Yponomeutidæ.

CONCLUSIONS

1. The two prothoracic glands in *Apantesis arge* are located under the patagia. The aperture of each is heavily pigmented, heavily chitinized, and thickly clothed with scales. The glands occur in both males and females.

2. The gland is a pouch formed by invagination. The epithelial lining is composed of very thin cells with large nuclei. There is no direct connection with the body cavity. Blood cells do not enter the lumen.

3. The secretion is a clear honey colored liquid, oily, and miscible with water. It forms a white precipitate in alcohol, gives no reac-

tion with litmus, turns black upon prolonged exposure to air, appears to be slightly granular, and possesses at times a slightly musty odor. It is not luminous.

4. The total secretion of an animal which had taken neither food nor drink was .012 cc. The maximum amount capable of being secreted at one time from one gland was .002 cc. The capacity of the gland is roughly .002 cc. Three to twelve hours are required to partially replenish the supply. The moths under observation did not secrete at the time of emergence and were capable of secreting till death. Secretion occurred only when the animal was disturbed.

5. The glands of adult Lepidoptera are probably protective devices. They occur widely in the Arctiidæ, and gland-like structures have been reported in Lasiocampidæ, Lymantriidæ, Zygænidæ, Noctuidæ, Notodontidæ, and Yponomeutidæ. They are probably present in other forms also.

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